DNA Looping and *Lac* Repressor–CAP Interaction

The promoter regions of both eukaryotic and prokaryotic genes that are regulated contain multiple binding sites for transcription factors (*1*). Interactions between and among regulatory proteins bound to promoter regions produce both positive and negative synergistic effects on gene expression in both eukaryotes and prokaryotes (*1*). The *lac* promoter of *Escherichia coli* has three binding sites for the *lac* repressor (*lac*R) centered at $+11(O_1)$, $+400(O_2)$, and $-82(O_3)$ relative to the transcription start site and one for the catabolite gene activator protein (CAP) centered at -61.5 (*2*). Lewis and his colleagues (*3*) proposed a detailed structural model based on co-crystal structures, with CAP bound inside a DNA loop stabilized by *lac*R bound to O_1 and O_3 on the opposite side of the DNA. We do not find this model to be correct.

The *lac*R is a tetramer and contains two binding sites for *lac* operator DNA (*4*, *5*). Our 2.6 Å resolution crystal structure of the *lac*R core protein (*6*) showed two *lac*R dimers held together by an antiparallel four-helix bundle of COOH-terminal α-helices, as suggested earlier by molecular genetic studies (*7*). Unanticipated, however, was the v-shaped, almost parallel arrangement of the two dimers that placed the DNA binding sites adjacent to each other on the same side of the tetramer (6). This arrangement of the binding sites has implications for the possible wrapping of multiple-operator–containing DNA that we explored (6) within the constraints of inter-operator spacing and footprinting data from Müller-Hill and his colleagues (*5*). Among the models considered (but not published because of the experiment described below) was the possibility that *lac*R (bound to O_3) and CAP [bound to its site 20.5 base pairs (bp) away] would interact favorably side by side, as has been demonstrated to occur in the Deo promoter, where CAP interacts with CytR (a repressor homologous to *lac*R), which is bound two turns of DNA away (*8*). Previously published work by Hudson and Fried (*9*) demonstrates cooperativity between *lac*R and CAP when they bind the *lac* operator region. However, when Hudson and Fried performed DNA footprinting experiments on ternary *lac*R-CAP-DNA complexes, they did not detect protection of O_3, although O_1 and CAP1 were efficiently protected.

To find out whether CAP1 and O_3 are bound cooperatively by their respective regulators, we performed gel retardation assays with a double-strand oligonucleotide spanning the corresponding region of the *lac* promoter (top sequence of Fig. 1A). We detected no ternary complex between *lac*R, CAP, and DNA, although either protein bound DNA efficiently to form the respective binary complex (Fig. 2A). When either of the binary complexes was combined with variable concentrations of the third protein, we observed competition for the DNA probe, present in limiting concentrations. We obtained similar results when we repeated these experiments with oligonucleotides bearing sequences of various affinities for CAP and *lac*R, separated by 20.5 bases (*10*). We designed an oligonucleotide that carried symmetrized versions of both *lac*R and CAP-binding sequences, identical to those used to solve the structure of the DNA complexes of these proteins (*3*, *11*), except for a five-base "overlap" (bottom sequence, Fig. 1A). These symmetric versions of the operators were spaced by 20.5 bp, as in the original *lac* operator, and the resulting DNA molecule mimicked part of the molecule that Lewis and his collaborators state was used in their model building of the ternary complex (*3*). No ternary complex was detected on the DNA molecule we designed, and competition between *lac*R and CAP occurred, as in the case of the original promoter sequence (Fig. 2B). Quantification of the binary complexes and free probe showed that the affinity of CAP and *lac*R for DNA was markedly reduced in the presence of an excess of the other protein, thus confirming our hypothesis that these proteins compete for binding to cognate sites separated by 20.5 bp, although they cooperate when the same sites are 72 bp away (*10*).

There are several other reasons to doubt that the detailed structural model of *lac*R and CAP bound to the *lac* promoter presented by Lewis *et al.* (*3*) is correct. First, because the CAP1 site on the *lac* promoter is centered at -61.5, the O_3 site

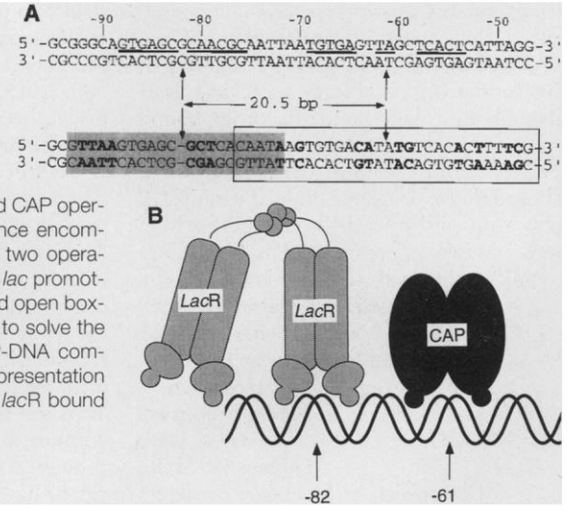

Fig. 1. (**A**) DNA sequences used in the gel retardation assay experiments of Fig. 2, A and B. Arrows indicate the center of symmetry of the *lac*R (base −82) and CAP (base −61.5) operators. Top sequence is from the *lac* promoter. Base numbers are according to Gilbert (*14*). Symmetric sequences of the *lac*R and CAP operators are underlined. Bottom sequence encompasses symmetrized versions of the two operators. Bases that are different from the *lac* promoter sequences are in bold. Shaded and open boxes indicate the DNA fragments used to solve the structure of the *lac*R-DNA and CAP-DNA complexes, respectively. (**B**) Schematic representation of the putative complex of CAP and *lac*R bound on O_3 and CAP1.

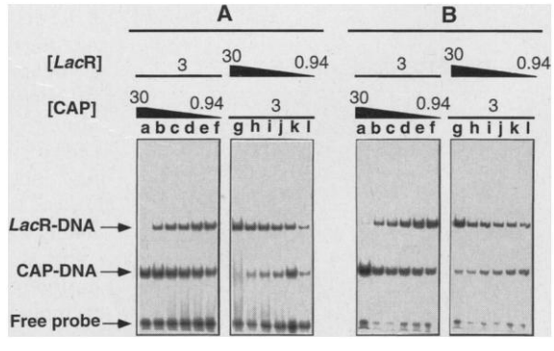

Fig. 2. Gel retardation assays with *lac*R and CAP. Top (**A**) and bottom (**B**), DNA sequences shown in Fig. 1A were end labeled and incubated with purified CAP and *lac*R [purification as described by Schultz *et al.* (*11*) and Friedman *et al.* (*6*), respectively]. Binding reactions performed in low ionic strength, essentially as described by Hudson and Fried (*9*); DNA concentration, approximately 1nM. Separation by PAGE, as described by Perros *et al.* (*15*). Lanes a to f: complexes formed in the presence of 3 nM *lac*R and CAP concentrations, as follows: (a) 30 nM; (b) 15 nM; (c) 7.5 nM; (d) 3.75 nM; (e) 1.88 nM; and (f) 0.94 nM. Lanes g to l: complexes formed in the presence of 3 nM CAP and *lac*R concentration, ranging from 30 nM to 0.94 nM.

is centered at −82 (Fig. 1A), and both proteins bind with their centers facing the minor groove, they must bind to the same side of the DNA (Fig. 1B). Lewis *et al.*'s model has the two proteins on opposite sides of the DNA resulting, it seems, from an incorrect connecting of the co-crystal structures of CAP and *lac*R complexed with DNA's that have five bp (−72 to −76) in common. Second, the positions of hypersensitive and protected deoxyribonuclease I (DNase I) cleavage sites in the DNA between O_1 and O_3 when bound to *lac*R (*5*) are not consistent with looping of the type proposed by Lewis *et al.* Third, the bend of bound operator DNA observed in the crystal structure, which might facilitate formation of the hypothesized loop, occurs only when the symmetrized DNA is missing the central base pair. Wild-type operators do not bend upon binding to *lac*R (*12*).

Although there is cooperative binding of CAP and *lac*R to DNAs of sufficient length, the cooperativity appears to be independent of the presence or absence of the O_3 sequence, but dependent on the length of the DNA upstream of O_3 (*13*). Fried and his co-workers (*13*) have suggested that cooperativity is achieved by CAP's ability to bend DNA and assist in the formation of a loop with one *lac*R dimer bound to O_1 and the other bound nonspecifically to DNA that lies more than 20 bp upstream of the center of O_3. Their data (*13*) suggest, indeed, that CAP may bind within a 140- to 160-bp loop between two segments of DNA that are bound to the *lac*R tetramer, but not the way that is proposed by Lewis *et al.* (*3*).

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REFERENCES AND NOTES

1. M. Ptashne, *Nature* **322**, 697 (1986); H. Echols, *J. Biol. Chem.* **265**, 14697 (1990); R. Schleif, *Annu. Rev. Biochem.* **61**, 199 (1992).
2. B. de Crombrugghe, S. Busby, H. Buc, *Science* **224**, 831 (1984); S. Oehler, E. R. Eismann, H. Krämer, B. Müller-Hill, *EMBO J.* **9**, 973 (1990).
3. M. Lewis *et al.*, *Science* **271**, 1247 (1996).
4. J. A. Borowiec, L. Zhang, S. Sasse-Dwight, J. D. Gralla, *J. Mol. Biol.* **196**, 101 (1987).
5. H. Krämer *et al.*, *EMBO J.* **6**, 1481 (1987).
6. A. M. Friedman, T. O. Fischmann, T. A. Steitz, *Science* **268**, 1721 (1995).
7. S. Alberti, S. Oehler, B. von Wilcken-Bergmann, B. Müller-Hill, *EMBO J.* **12**, 3277 (1993).
8. L. Søgaard-Andersen and P. Valentin-Hansen, *Cell* **75**, 557 (1993).
9. J. M. Hudson and M. G. Fried, *J. Mol. Biol.* **214**, 381 (1990).
10. A. M. Friedman, T. O. Fischmann, T. A. Steitz, unpublished data.
11. S. C. Schultz, G. C. Shields, T. A. Steitz, *Science* **253**, 1001 (1991).
12. S. Zinkel and D. M. Crothers, *Nature* **328**, 178 (1987); H.-M. Wu and D. M. Crothers, *ibid.* **308**, 509 (1984).
13. K. M. Vossen, D. F. Stickle, M. G. Fried, *J. Mol. Biol.* **255**, 44 (1996).
14. W. Gilbert, in *Protein-Ligand Interactions*, H. Sund and G. Glauer, Eds. (de Gruyter, Berlin, 1975), p. 193.
15. M. Perros *et al.*, *J. Virol.* **69**, 5506 (1995).
16. We thank T. Fischmann for help in preparing *lac*R and C. Scafe for a gift of CAP. M.P. was supported by a postdoctoral fellowship from the International Human Frontier Science Program Organization.

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The lactose operon has long served as a paradigm for gene expression and its regulation. The recent solutions by Lewis and his colleagues of the crystal structures of the *lac* repressor and its complexes with DNA and isopropyl-β-D-1-thiogalactopyranoside (IPTG) provide highly detailed views of molecular interactions that control the *lac* system (*1*). These structures also provide a rational basis for the construction of models of regulatory complexes that contain additional proteins. In one such model, proposed by Lewis and his colleagues, a *lac* repressor tetramer forms a bridge between operator sites O_1 and O_3, and the intervening DNA (approximately 90 bp) forms a smoothly curved loop. The structure of the transcription activator protein (CAP) complex with bent DNA, determined by Schultz *et al.* (*2*), was built into Lewis *et al.*'s model, with its DNA occupying the position corresponding to the principal CAP site (*C1*) of the *lac* promoter. Lewis *et al.*'s model accounts, in principle, for the enhanced repression of *lac* transcription observed when templates contain site O_3 as well as O_1 (*3*). Although a role for CAP in this effect has yet to be shown, its ability to bend DNA by 90° to 130° (*2*, *4*) makes its inclusion plausible.

Lewis *et al.*'s model requires the simultaneous binding of site *C1* by CAP and of O_3 by repressor, with the proteins occupying opposite faces of the DNA helix. Data currently in the literature *appear* to argue against this. First, when CAP and repressor bind independently, their sites, defined by DNase I protection, overlap by approximately 9 bp [(*5*); compare also Fig. 1, lanes c and g]. Second, the center-to-center separation of these sites is only 20.5 bp [see, for example (*6*, *7*)]. If the intervening DNA is B-form, both proteins should occupy the same face of the helix. These results predict steric clash between CAP and repressor that might prevent simultaneous binding

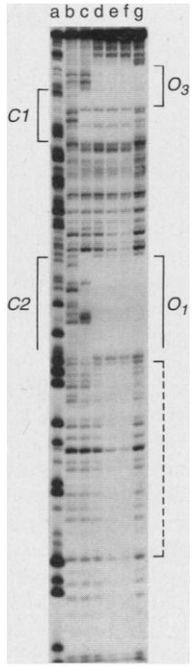

Fig. 1. DNase I footprint analysis of the interactions of CAP and *lac* repressor with *lac* promoter DNA. The DNA used was a 204 bp HinfI-PvuII fragment, 5′ ^{32}P-labeled on the template strand; binding and footprinting conditions were as previously described (*5*). Lane a, G > A sequencing reaction (*11*) products. Lanes b to g contained 2.1 nM *lac* DNA. Lanes c to f, 4.1 nM CAP; lanes d to g contained 4.5, 13.6, 22.7, and 6.3 nM *lac* repressor, respectively. CAP footprints at sites 1 and 2 labeled *C1* and *C2*; those of *lac* repressor at operators 1 and 3 labeled O_1 and O_3.

and a geometry for the *C1*-O_3 complex that is different from that proposed by Lewis *et al.* (*1*).

We performed DNase I footprinting to test these notions (Fig. 1). Protein-DNA stoichiometries of individual complexes were determined by mobility-shift assay [(*8*); results not shown]. The binding of two CAP dimers resulted in the protection of sites *C1* and *C2* (lane c); the binding of two *lac* repressor tetramers resulted in the protection of sites O_1 and O_3. These protection patterns correspond well with ones in the literature (*5*, *6*). The protection patterns shown in lanes d through f are consistent with the simultaneous binding of CAP and *lac* repressor in the *C1*-O_3 region. However, when both proteins are bound, the margin of DNase protection in the repressor site is moved upstream by 6 bp (*9*). This interval is nearly the same as that of the *C1*-O_3 overlap (see above). Displacement of repressor by 6 bp should therefore reduce the potential for steric clash with CAP and place the proteins on opposite faces of a B-DNA helix, as required by Lewis *et al.*'s model (*10*). In view of the high density of protein binding sites found in promoters, assembly mechanisms that involve binding site isomerization, like the one shown here, may represent a common theme in transcriptional regulation.

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REFERENCES AND NOTES

1. M. Lewis *et al.*, *Science* **271**, 1247 (1996).
2. S. C. Schultz, G. C. Shields, T. A. Steitz, *ibid.* **253**, 1001 (1991).
3. S. Oehler, M. Amouyal, P. Kolkhof, B. von Wilcken-Bergmann, B. Muller-Hill, *EMBO J.* **13**, 3348 (1994).
4. S. Zinkel and D. Crothers, *Biopolymers* **29**, 29 (1990).
5. J. M. Hudson and M. G. Fried, *J. Mol. Biol.* **214**, 381 (1990).
6. A. Schmitz, *Nucleic Acids Res.* **9**, 277 (1981).
7. W. Gilbert, J. Majors, A. Maxam, in *Dahlem Workshop on Organization and Expression of Chromosomes*, V. Allfrey, E. Bautz, B. McCarthy, R. Schimke, A. Tissieres, Eds. (Abakon, Berlin, 1976), pp. 167–176.
8. M. Fried and D. Crothers, *Nucleic Acids Res.* **9**, 6505 (1981); ———, *J. Mol. Biol.* **172**, 241 (1984).
9. The sequence of the displacement site lacks obvious homology with known operators, and we have no evidence for its occupancy by repressor in the absence of CAP.
10. The downstream margin of DNase protection at site C1 and its characteristic hypersensitive sites were unchanged, suggesting that the CAP-DNA interaction is not perturbed by repressor binding at O_3. In contrast, in the C_2-O_1 region, the CAP footprint was replaced by that of repressor and a diffuse region of protection appeared downstream of O_1. A detailed analysis of these effects will be presented elsewhere.
11. A. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).

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Response: For many years the repressor of the lactose operon has served as a prototypical model for studying gene regulation. The x-ray structures that we reported (*1*) of the repressor; the repressor bound to an allosteric effector, isopropyl-β-D-1-thiogalactoside; and the repressor complexed to a symmetric 21-bp operator, provide atomic models that describe the conformation of the repressor in the induced and the repressed states. By comparing the three structures, we could describe the transition between states. Interestingly, the allosteric changes are fundamentally analogous to the transition between the T and the R states observed in hemoglobin (*2*). The structural transformation, however, is localized and requires only a dimeric repressor, even though the observed quaternary structure is tetrameric. From the work of Müller-Hill and his coworkers, it is clear that the intact tetramer is required for maximal repression (*3*), and high levels of repression are observed only when the tetrameric repressor binds to its primary operator, O_1, and an ancillary site, either O_3 (93 bp upstream from O_1) or O_2 (401 bp downstream), and thereby form repression loops (*4*). We built a model of the tetramer bound simultaneously to O_1 and O_3 in order to account for the observed tetrameric repressor and to provide a physical representation of these repression loops. Our model was constructed by creating the intervening DNA with a radius of curvature of about 40 Å, thereby linking the O_1 and O_3 sites. Perros and Steitz raise objections to this model and argue against the proposed looping of the repressor.

When the repressor associates with a symmetric operator, there is a distinct bending of the DNA, and a pair of hinge helices fit into the minor groove analogous to that observed with dimeric purine repressor (*5*). The binding to the minor groove, by necessity, distorts the DNA. The minor groove widens, which causes the operator to bend away from the repressor. Perros and Steitz suggest that the bending of the DNA is an artifact of using a symmetric sequence and propose that the repressor does not alter the conformation of the wild-type operator DNA. In contrast, the genetic analysis of Miller and his co-workers has shown that mutations in the hinge helix alter the ability of the protein to repress transcription (*6*). A large number of amino acid substitutions within the hinge helix result in repressor molecules that can no longer bind to the operator. In fact, the hinge helix is nearly as sensitive to mutation as the amino acid substitutions in the recognition helix, suggesting that it plays a crucial role and most likely binds to the DNA, as seen in both the x-ray and the nuclear magnetic resonance structures (*1*, *7*). Moreover, the hinge helix transmits the allosteric signal from the inducer binding site to the DNA binding domain. While the structural studies are admittedly contrary to the results of Crothers and his co-workers (*8*), they are fully consistent with the position-dependent bending observed by Adhya and his co-workers (*9*).

Perros and Steitz also argue that the hypersensitivity and protected DNase I cleavage sites as seen by Krämer *et al.* (*10*) are inconsistent with the looping model. These experiments demonstrated the importance of correctly phasing the two operators and showed that the position of enhanced and decreased DNase I sensitivity depends on the number of base pairs separating the two operators. Because the spacing of O_1 and O_3 is much larger than those used in the experiments, and because the intervening sequence was nonnative, it seems quite difficult to extrapolate and predict what will happen with the natural operon.

Using gel retardation assays, Perros and Steitz demonstrate that the repressor and the catabolite gene activator proteins (CAPs) cannot bind simultaneously to a fragment of DNA that encompasses both the O_3 and the CAP1 sites. The bindings of the two proteins are mutually exclusive, and competition results when the sites are 20.5 bp apart; however, there is cooperativity between CAP and *lac* when the separation is 72 bp (*11*). Although the information obtained from these experiments is useful, the DNA fragment is too short for mutual binding; therefore, their design does not address the issue. The correct experiment, which is central to our model, is described in the comment by Hudson and Fried. It is clear from their results that, given an appropriate length of DNA, repressor can bind both O_1 and O_3 in the presence of CAP. Moreover, CAP increases the affinity of the repressor for its sites. In our opinion, the results of Perros and Steitz support our model by demonstrating that repressor and CAP cannot bind on the same face of the DNA. Repressor and CAP can and do bind simultaneously to O_3 and the CAP1 sites, as seen in the footprinting gel of Hudson and Fried. The apparent discrepancy is resolved by noting that the repressor contact site is moved upstream by 6 bp, which places the interaction on the opposite face of the DNA from CAP.

When the *lac* repressor binds two distinct operators, it is likely that repression loops will form with a structure that depends on the physical properties of DNA, as well as the length of the intervening loop (*12*). Forming stable looped complexes, particularly for relatively short stretches of DNA, may require additional DNA binding proteins that can dramatically change the physical properties of the nucleic acid. CAP, for example, interacts with a DNA sequence between the primary and an ancillary O_3 site. CAP binding induces a bend of approximately 90° over about 30 bp of DNA, causing the DNA to kink (*13*). In our model, the CAP assists the repressor in forming the repression loop. While it may seem paradoxical that a transcriptional activator (CAP) would stabilize or promote the repressed state, loops between O_1 and O_3 most likely form when there are low concentrations of glucose (and therefore elevated concentrations of cyclic AMP promoting CAP binding) and lactose. In such instances, the bacterium needs to reduce the basal level of transcription of the *lac* operon to conserve energy, which is consistent with diauxic growth (*14*). In this way, the *lac* repressor and CAP operate as an integrated switch that responds to the relative concentration of these metabolites. Our proposed physical model of the repression loop accounts for the vast amount of experimental data describing the regulation

of the lactose operon. In our view, the model is fundamentally correct and consistent with experimental results.

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REFERENCES

1. M. Lewis *et al.*, *Science* **271**, 1247 (1996).
2. M. F. Perutz, W. Bolton, R. Diamond, H. Muirhead, H. C. Watson, *Nature* **203**, 687 (1964).
3. S. Oehler, E. R. Eismann, H. Krämer, B. Müller-Hill, *EMBO J.* **9**, 973 (1990).
4. J. A. Borowiec, L. Zhang, S. Sasse-Dwight, J. D. Gralla, *J. Mol. Biol.* **196**, 101 (1987).
5. M. A. Schumacher, K. Y. Choi, H. Zalkin, R. G. Brennan, *Science* **266**, 763 (1994).
6. P. Markiewicz, L. Kleina, C. Cruz, S. Ehret, J. H. Miller, *J. Mol. Biol.* **240**, 421 (1994).
7. C. A. E. M. Spronk, M. Slijper, J. H. van Boom, R. Kaptein, R. Boelens, *Nature Struct. Biol.* **3**, 916 (1996).
8. H.-N. Liu-Johnson, M. R. Gartenberg, D. M. Crothers, *Cell* **47**, 995 (1986).
9. C. J. Zweib, J. Kim, S. Adhya, *Genes Dev.* **3**, 606 (1989).
10. H. Krämer, M. Amouyal, A. Nordheim, B. Müller-Hill, *EMBO J.* **7**, 547 (1988).
11. M. J. Hudson and M. G. Fried, *J. Mol. Biol.* **214**, 381 (1990).
12. G. R. Bellomy, M. C. Mossing, M. T. Record, *Biochemistry* **27**, 3900 (1988); M. C. Mossing and M. T. Record Jr., *Science* **233**, 889 (1986).
13. S. C. Schultz, G. C. Shields, T. A. Steitz, *Science* **253**, 1001 (1991).
14. J. Monod, thesis, Université de Paris (1942).

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The Loss of Atmosphere from Mars

Luhmann *et al.* (*1*) and Jakosky *et al.* (*2*) showed that the Martian atmosphere was eroded (sputtered) by energetic O^+ ions that are formed from escaping O and accelerated back into the atmosphere by the solar wind fields. This collisional ejection process appears to explain measured isotope ratios for Ar and N in the martian atmosphere (*2*, *3*) and it may affect the early evolution of this atmosphere (*1*–*3*). More recently, D. M. Kass and Y. L. Yung (*4*) presented a more detailed calculation of the loss of Martian atmosphere. They found that 3 bars of CO_2 are driven off by sputtering, an amount three times greater than the size of Earth's atmosphere. This is a huge increase in atmospheric loss over the earlier estimate of about 0.1 bars (*1*, *2*). This increase came about because Kass and Young assumed that full dissociation of CO_2 ($\rightarrow$ C + 2O) occurs readily in collisions of an incident O with CO_2. Therefore, C atoms, which have much lower gravitational escape energies than CO_2 or CO, are efficiently formed *and* energized, which increases the loss of C dramatically.

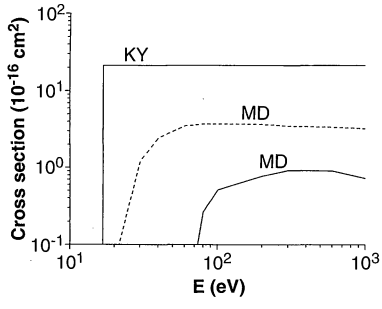

Fig. 1. Dissociation cross sections for O + CO_2 collision plotted as a function of the energy of the O atom. Solid lines: O + CO_2 $\rightarrow$ O + C + 2O; dashed line: O + CO_2 $\rightarrow$ O + CO + O. Line labelled KY, cross section assumed by Kass and Yung (*1*); curves labelled MD, calculated values using molecular dynamics with the universal interaction potential (*6*) for the interaction of the energetic O with individual atoms in CO_2. Three pair potentials are used for CO_2, which gives the correct dissociation energy for CO_2 and for the resulting CO.

Because the collisional dissociation cross sections in the energy range of interest (~20 eV to 1 keV) have not been measured, the dissociation cross section used by Kass and Yung essentially maximized the atmospheric loss process. The cross section they used for dissociation in O + CO_2 collisions can be compared to a molecular dynamics calculation (Fig. 1). In that calculation, the energetic O interacts with each of the atoms in the molecule that are bound together by pair potentials chosen to reproduce the binding energies and interatomic separations of CO_2 and the dissociation product CO. Although the use of pair potentials in this manner typically leads to an overestimate of the dissociation cross section, the threshold for full dissociation (solid curves) described by Kass and Yung is shifted by about a factor of 5 from that calculated, and the size of their cross section is more than an order of magnitude larger than that calculated. Because the *size* of their cross section is roughly that of the elastic collision cross section, the net contribution of dissociation to the atmospheric loss process is more than an order of magnitude too large. In addition, the primary collisional dissociation channel is seen to be CO_2 $\rightarrow$ O + CO, so that only a small fraction of the struck CO_2 produces C atoms. Therefore, although it is correct that including CO_2 dissociation in *all* stages of the cascade of collisions initiated by an incident O^+ increases the C loss rate over that described earlier (*1*, *2*), Kass and Yung's estimate of the effect is an order of magnitude too large.

Although over the history of Mars it is certainly possible that more atmosphere may be driven off by sputtering than the amount given by Luhman *et al.* (*1*) and by Jakosky *et al.* (*2*), it cannot occur in the manner suggested by Kass and Yung (*4*), even if their cross sections were correct. That is, as the atmospheric escape rate increases, the region in which the solar wind ionizes *and* accelerates the escaping atoms occurs at larger distances from the planet (*5*), reducing the fraction of these ions that impact the atmosphere. In the earliest martian epoch this feedback process is already problematic for the much lower escape rates calculated by Luhman *et al.* (*1*) and by Jakosky *et al.* (*2*).

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REFERENCES

1. J. G. Luhmann, R. E. Johnson, M. H. G. Zhang, *Geophys. Res. Lett.* **19**, 2151 (1992).
2. B. Jakosky, R. O. Pepin, R. E. Johnson, J. L. Fox, *Icarus* **111**, 271 (1994).
3. R. O. Pepin, *ibid.*, p. 289.
4. D. M. Kass and Y. L. Yung, *Science* **268**, 697 (1995).
5. J. G. Luhmann, *Space Sci. Rev.* **44**, 241 (1986).
6. R. E. Johnson, *Energetic Charged Particle Interactions with Atmospheres and Surfaces* (Springer, Berlin, 1990).

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Response: The results presented in our report (*1*) indicated that it was necessary to consider dissociation during all collisions in calculating the atmospheric loss from Mars that results from sputtering. With the use of the newly calculated cross sections presented by Johnson and Liu in our model, we find that Mars has lost about 1 bar of CO_2. The revised cross sections reduce our sputtering yields (Table 1), but do not bring our results into agreement with Luhmann *et al.* (*2*) and Jakosky *et al.* (*3*).

The effective decrease in the collisional cross section pointed out by Johnson and Liu of CO_2 $\rightarrow$ CO + O (channel I) by a factor of about 5 and of CO_2 $\rightarrow$ C + 2O (channel II) by a factor of about 50 will not result in decreases of 5 and 50, respectively, in the collision frequency with CO_2. At the important energies for sputtering, collisions with CO_2 result in some form of dissociation (Table 1).

The changes in the cross section (a factor of 5 for channel I and a factor of 10 for channel II) do not have a linear effect on the collision probability, but the ratio of